Synthesis and Characterization of Silica Microcapsules with Active Compounds 2% Chlorhexidine Using Sodium Alginate and Chitosan Coating as Medicament of Root Canal Infection

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Abstract

Enterococcus faecalis is a leading cause of bacterial persistence root canal infections. These bacteria have the ability to invade dentinal tubule size which is very small between 1-3μm causing difficulty drug penetration to eliminate root canal. 2% Chlorhexidine is recommended as a remedy to eliminate root canal bacteria. Root canal drugs should remain stable in solution and remain active despite the blood, serum and tissue protein derivatives. Necessary efforts to protect the root canal medication microencapsulation ahould be made. In this research, 2% chlorhexidine microencapsulation process measures less than 1 μm by using sodium silicate as a precursor. The method used is solgel with the Stober process. Clustering using chitosan and sodium alginate material made to encapsulate 2% chlorhexidine in higher numbers. The purpose of this study was to obtain silica microcapsules with active compounds 2% chlorhexidine the size of less than 1 μm homogeneously and analyze its release rate at pH 6.5 as the pH of the inflamed root canal. The evaluation of the succession of the microencapsulation process is characterization of SEM, FTIR and BET, while the rate of spending chlorhexidine (release rate) of the microcapsules is calculated using a UV-Vis spectrophotometry. The results showed that the microcapsules measures less than 1 μm with a release rate of 0.0286 ppm per minute for 55 minutes.

Keywords

Microencapsulation; Chlorhexidine

Introduction

Enterococcus faecalis is bacteria commonly found in teeth root canal and remains inside even after a treatment. This bacteria are responsible for the infection of root canal for 80 – 90 %. It is usually the only Enterococcus species isolated from root canal which has completed a treatment.(Fisher & Phillips, 2009) *Enterococcus faecalis* has an ability to invade dentinal tubules, which isn't inclusive in all bacteria. Dentinal tubules have very small size, which makes the penetration of root canal drug difficult in eliminating it. *Enterococcus faecalis* can enter the phase of Viable but Non Culturable (VBNC). It is the phase where bacteria can survive even though it does not breed.(Lleo, 2001) This bacteria have an ability to survive in an environment with high pH and it can also survive as an organism in root canal that can invade the dentinal tubules. Thus, it makes the *Enterococcus faecalis* become a pathogenic bacteria and can cause a failure in the treatment of root canal.(Molander et al, 1998; Love, 2001; Portenier et al, 2003; Sundqvist & Fidgor, 2003) Virulance factor such as *lipotheichoic* acid (LTA), hemolysin, gelatinase and aggregation substance has an important role in pathogenesis. The bacteria contaminate root canal and form a colony on dentin surface by the help of LTA, while the aggregation substance and other surface adhesion contribute to the attachment in collagen. Cytolysin, AS-48 and bacteriosin which are the products of this bacteria, are capable of inhibiting the growth of other bacteria. This shows the small amount of other bacteria in a persistent root canal infection, so the *Enterococcus faecalis* becomes a dominant microorganism in root canals.(Kayaoglu & Ørstavik,

2004) 2% chlorhexidine has been recommended as a root canal drug. It is usually used to kill germs, bacteria, or even fungi. At pH 5,5-7,0, chlorhexidine will bond with negative content on bacterial cell wall. Low concentration chlorhexidine will cause the change in the balance of osmosis pressure of the bacteria cell and also cause leakage of potassium and phosphorus, whereas at high concentration, chlorhexidine will cause the content of cytoplasm of the bacteria cell to settle and eventually leads to cell death.(Lennet et al, 2000; Ryan, 2010)

There are some requirements that must be owned by root canal drug. One of them is, the root canal drug should remain stable in solution and remain active as well even in blood, serum, and in tissue protein derivatives.(Grossman et al, 1995) Based on that, effort is needed in order to protect the root canal drug from the influence of its environment. Microencapsulation is an effort to protect the drug from the influence of its environment and also serves to regulate the drug release through controlled release mechanism. The technology of Drug Delivery System (DDS) has been a developing market in the world nowadays. In the encapsulation process, there is a process of the formation of the outer layer (coating) of capsule wall using protective materials. Due to its function as a protector, the non existence of interaction between the core and the coating layer is expected, and the encapsulation itself will protect the core from the environment condition, enhance the stability, extend the life of the core, and regulate the release rate of active substances.(Kailasapathy, 2002) The clustering process is based on the phenomenon of the kidney stone formation in human body which is caused by saturated urine and its constituent salts, such as calcium oxalate and uric acid that bond with Hyaluronic Acid (HA) and they cannot be excreted through urine. In the process of the formation of kidney stone, HA plays a role in determining the urine concentration, inhibiting crystallization, crystal storage. (Verkeolen, 2007) An analysis is conducted to HA on the natures and the working active clusters during the clustering process. The clusters are carboxyl (C-O-OH), hydroxyl (C-OH), and amine (N-H_2). In this study, clustering material which is not used is Hyaluronic Acid (HA). The materials used are the materials having the same natures and active clusters, so is used for making microcapsules because silica has a porous structure that can be penetrated by active substances and is suitable to be used as DDS material.(Kleitz, 2009) Silica also has biocompatible, biodegradable, and nontoxic natures, which makes it safe to use.(Kleitz, 2009; Patel, 2010)

Chitosan is a polysaccharide derived from the refined acetyl (deacetylation) of chitin material. It is the second highest biopolymer material in nature after cellulose. Chitin is a common material found in animals with external bones, such as arthropods, crustaceans, insects, and molluska.(Kumar, 2005) Various advantages owned by chitosan, make it is widely used in medical field. The material has biocompatible and biodegradable natures and is available in abundant quantities. (Couto et al, 2009) In chitosan, the chains susceptible for reaction are amine free cluster and hydroxyl cluster. In certain circumstances, various types of reactions can be set by modifying amine cluster.

Alginate is one of the most suitable biopolymers for microcapsule application. Its composition and structure have suitable function and role as the encapsulation material. (Finotelli et al, 2010) In the alginate, the chain susceptible for reaction is active carboxyl cluster. Thus, by the presence of alginate and chitosan, the active cluster and the nature owns by hyaluronan can be obtained. In market, alginate is widely available in the form of sodium alginate.

There are two ways to produce nanomaterial, i.e. top down and bottom up. Top down means breaking a big material and making it into small-sized material using mechanical force, chemical reaction, or other energy. Bottom up means the synthesis of atom or molecule using a chain reaction, making into nano-sized. (Luther, 2004) This study uses the bottom up method with sol-gel process. Sol-gel process is described as the formation of oxide bond through precursor molecule polycondensation reaction in liquid media. Generally, sol process involves the transition phase of sol liquid into solid gel. (Bush, 2006) One of the sol gel method is stober methods. Stober process, discovered by Werner Stober in 1968 and obtained from the chain reaction of silica precursor with alcohol liquid and alkali catalyst water, is used to obtain evenly dispersed silica particles. (Stober et al, 1968) The regulation of porosity of microcapsules will make the diffusion of encapsulated active compounds controlled. (Franjione & Vasishtha, 1995) Synthesis of silica microcapsules and 2% chlorhexidine active compound less than 1 μm in size and clustering of some microcapsules formed by using chitosan and sodium alginate will be

conducted in this study as an effort to entrap the active compound in greater numbers, but the result of the clustering will remain less than 1 μm in size.

Methodology

The study uses experimental method. Some samples used are differentiated based on the chitosan and alginate compositions. The procedure used is solgel with Stober process.

Making the Precursor

The precursor used is sodium silicate 0,5 M. The precursor is derived from liquid sodium silicate dissolved in distilled water until the concentration reaches 0,5 M. Furthermore, the solution is stirred using magnetic stirrer for 10 minutes.

Making the Stober Solution

Ethanol 150 ml is used as the solvent. 15 ml of distilled water and ammonia 25% for 3 ml are added to the prepared ethanol. The ammonia added acts as a catalyst. After all solutions are mixed, 9 ml sodium silicate is added bit by bit.

Silica Microcapsule Synthesis

After the precursor and the Stober solution have been mixed, emulsification is then performed using the ultraturrax by 10.000 rpm in speed for 10 minutes. In the emulsification condition, chitosan 1% is added for 0,7% out of the total solution's volume. Next, for homogenization, ultrasonic homogenizer is used for 15 minutes. After the process is completed, the next step is the drying process using a vacuum machine until it can get dry silica microcapsule powder. The dried silica microcapsule powder is immersed in 2% chlorhexidine for 24 hours to make the chlorhexidine get in and trapped in the silica microcapsule. After 24 hours, the silica microcapsule is re-dried using a vacuum machine. Scanning Electron Microscope (SEM) characterization is performed to the result in order to find out the silica morphology obtained.

Silica Microcapsule Encapsulation

Encapsulation stage 2 is performed using chitosan and sodium alginate. The expected result is that the silica will gather forming a cluster so the release rate of substances will take longer. 0,1 gram of silica microcapsule is dissolved in 25 ml of distilled water and mixed with chitosan 2%, sodium alginate 2% and Ca2+ 0,01 M.

Result and Discussion

To find out how the morphology is formed, SEM characterization is performed. The SEM test is conducted using JSM-6510LA machine. Based on the SEM characterization result, silica microcapsule having less than 1 μm particle size is obtained. The particle size obtained depends on the used precursor, the alcohol media type, and the volume ratio. It can be seen in FIG. 1 that the resulting particles are dispersed and evenly scattered.

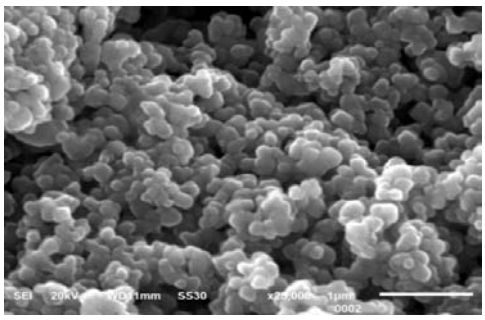

FIG. 1 SILICA MICROCAPSULE

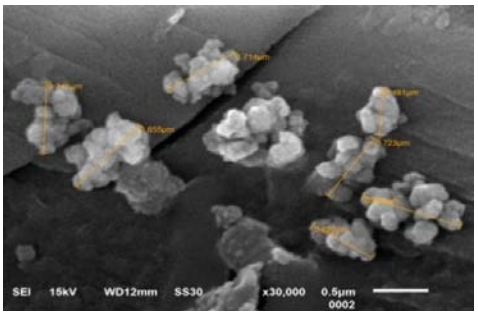

FIG. 2 SILICA MICROCAPSULE ADDED BY CHITOSAN 2% FOR 6% OF THE VOLUME, 0,5% SODIUM ALGINATE FOR 2% OF THE VOLUME, AND 2 ML CA^{2+} 0,01 M

This is in line with the study conducted by Werner Stober in 1968. In the study, the formed silica is the result of the sol-gel method with sodium silicate precursor. Furthermore, variance of chitosan and sodium alginate composition are given to the samples and to the next ones.

$$Na_2SiO_3 + 3\,H_2O \rightarrow Si(OH)_4 + 2\,NaOH \ldots.(1)$$

$$Si(OH)_4 + Si(OH)_4 \rightarrow Si - O - Si + H_2O \ldots(2)$$

As described previously, the clustering process is based on the formation of kidney stone by hyaluronic acid (HA) in human body. In this study, the function of HA is replaced by the presence of chitosan and sodium alginate. The basis of this study is some compositions needed and the order of mixing, so cluster form can be obtained. By the right composition, the optimum condition can be obtained (the silica is

coated and gathers forming a cluster) and it is potential as a good media for drug delivery.

FIG. 2 shows the formed clusters, and among the clusters there is a sized cluster less than 1 μm (approximately 0,4-0,9 μm). But, there are also the rest of uncovering chitosan. This is due to the uneven mixing process which uses only ultraturrax for 5 minutes and magnetic stirrer for 30 minutes resulting a weak collision between the silica and the chitosan. Chitosan will encircle silica (Si-O-Si) due to the interaction of the hydrogen bond and Si-O-C bond. Furthermore, amine cluster from the surrounded chitosan will react with other added compounds.

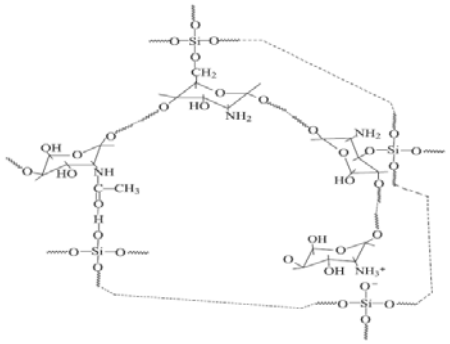

FIG. 3 THE BOND BETWEEN SILICA AND CHITOSAN

(Silva et al, 2011)

Chitosan will attach to silica particles due to the hydrogen bond between silanol in silica bond with amine and oxy in chitosan (FIG. 3). Sodium alginate has the ability to make a cross link by the presence of cation, like Ca^{2+}, and the alginate will form a reticulated structure as the result of interaction with Ca^{2+}. A strong electrostatic interaction from the amine in chitosan with carboxyl in alginate will create a complex chitosan-alginate bond, resulting the microcapsule more resistant to active substance release.(Kumar, 2005)

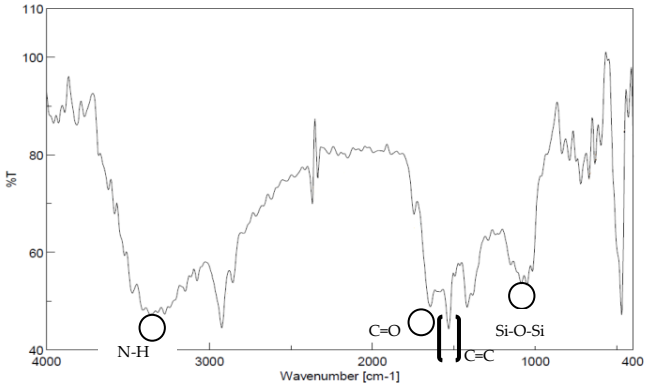

FIG. 4 FTIR CHARACTERIZATION RESULT OF SILICA MICROCAPSULE-CHITOSAN-ALGINATE BOND WITH 2% CHLORHEXIDINE

In Fourier Transform Infrared Spectroscopy (FTIR) test, there are elements of silica, alginate, chitosan, even the bond of those three elements. It makes the silica-alginate-chitosan synthesis as microcapsule material successfully formed. Peak at a frequency of 1091 cm^{-1} indicates the bond Si - O - Si. This suggests that the expected silica had been completely formed by Stober. Peak at a frequency of 1650.77 cm^{-1} indicating the presence of the C = O. It is identical to the formed carboxylic acid for, that carboxylic acids can be derived from the remains of chitin chitosan or alginate having ties carboxylic. Peak at a frequency of 3444 cm^{-1} indicates the bond O - H, illustrating the hydrogen bonding. Hydrogen bond from the bond between the silica and chitosan

Furthermore, test on microcapsule given 2% chlorehixidine active substance is carried out, and then it is observed whether or not the aromatic ring chain constituting 2% chlorhexidine has emerged. Based on the FTIR result, it can be seen in figure 4 that there is a peak in the frequency of 1531,2 cm^{-1} which shows the function C = C aromatic. By the presence of peaks, it can be assured that silica-chitosan-alginate microcapsules bond with 2% chlorhexidine have been formed.

TABLE 1 BET CHARACTERIZATION RESULT

Samples name	Spesific surface area	Total pore Volume	Average Pore Diameter
Silica	8,168m^2/g	3,497 e-02 cc/g	1,71250e+02 Å

Brunauer-Emmett-Teller (BET) characterization results showed silica microcapsule having a specific surface area of 8.168 m^2/g with an average pore diameter of 1.71250 e +02 Å

Time Release of Active Compound

To find out the level of 2% chlorhexidine release, Ultraviolet-Visible Spectroscopy (UV-VIS) characterization is used, by using the amount of absorbance of the checked samples after and before the length of maximum wave of the preferred material can be detected. In this study, it is chlorhexidine. The samples are dissolved in kokubo solution. Kokubo is one of the types of artificial body fluid (Simulated Body Fluid) which possesses a difference in terms of Cl^- and HCO_3^- content from human body fluid. The results of checked maximum wave length are 3 peaks; 209 nm, 231 nm, and 254 nm (FIG. 5). The appearance of 3 peaks is due to the checked samples which comprises several elements such as alginate, silica and

2% chlorhexidine. The wave length of 2% chlorhexidine is 254 nm and from the wave length checking has shown appropriate results.

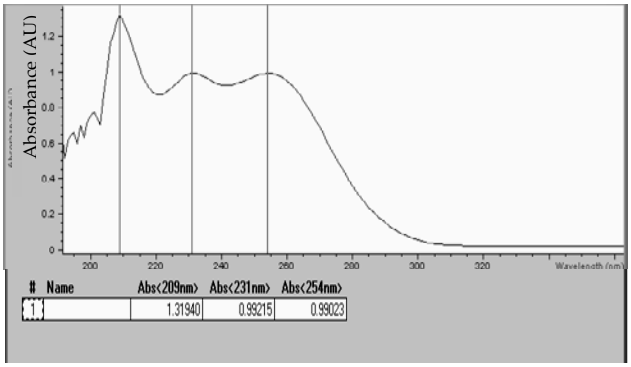

FIG. 5 LENGTH OF MAXIMUM WAVE

Furthermore, a test is carried out to shoot the 254 nm wave length. Measurement of absorbance value is counted in 5 minute range for 1 hour. To change the absorbance value into concentration value is performed by using the Beer-Lambert equation that indicates the absorbance value is directly proportional to the concentration value or by using the equation derived from standard solution. This study uses standard solution, so the absorbance value can be alternated into concentration value. In the first test, absorbance value in kokubo solution with normal pH of 7,4 is measured. In the second test, it will be performed in kokubo solution with inflammation pH of 6,5.

The test result on kokubo solution is shown in figure 6, The kokubo with pH 7,4 describes the condition of body fluid in healthy condition. When microcapsules containing chlorhexidine is inserted,the excretion of chlorhexidine from the microcapsules is observed. In the first five minutes, there is an excretion for 0,2 ppm, but after that, it tends to get stable. If it is seen more clearly, the excretion of chlorhexidine at pH 7,4 happens for 45 minutes with average rate 0,002 ppm per minute.

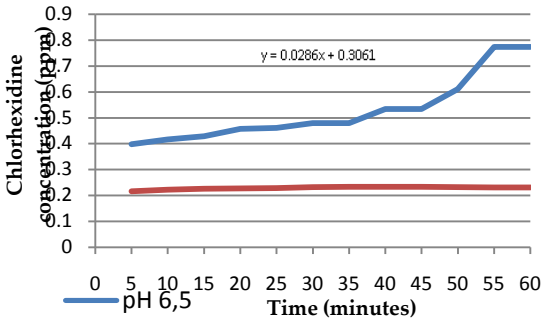

FIG. 6 THE TIME RELEASE OF 2%CHLORHEXIDINE

The low rate of excreted chlorhexidine will work at pH 5,5-7 and the environment condition during the test is 7,4. Imperfect encapsulation process can be a reason why chlorhexidine can be detected at pH 7,4.

In ideal encapsulation, all chlorhexidine will be trapped in the microcapsules, but it is still possible that there is chlorhexidine bonded with surface or contained in microcapsule pores. The part of chlorhexidine which is not in the microcapsules may lead to the detection of chlorhexidine at pH 7,4.

In kokubo solution at pH 6,5, it is seen that the tested samples have succeeded in excreting 2% chlorhexidine with the greater amount and concentration. The time release of chlorhexidine reaches 55 minutes with excretion average rate of 0. 0286 ppm per minute. The excreted rate of chlorhexidine increases to 10 times, and acidity becomes the main factor for the excretion of active substance. In acid condition, the chitosan coating the microcapsules tends to swell.(Ziwei et al, 2011) The swelling will lead to the opening of enlarged microcapsule pores, so it makes the chlorhexidine come out more. After 55 minute excretion, chlorhexidine tends to get stable. The stability arises due to the absence of chlorhexidine that comes out from the microcapsules. It is caused by the depleted chlorhexidine or the environment condition which is no longer supports the excretion of active substance.

2% chlorhexidine has a half-life or 60 minute-drug work period. (Shafiullah, 2005) The time release of chlorhexidine reaches 55 minutes. This shows that the resulted microcapsules have already given a pretty good efficiency. With the same amount, chlorhexidine without encapsulation will work for 60 minutes, while chlorhexidine encapsulation will work 55 minutes longer.

Conclusions

Silica microcapsules with 2% chlorhexidine active compound coated by sodium alginate and chitosan may be less than 1 μm in size, making it possible to get it in and function in dentinal tubules of teeth root canal and also 2% chlorhexidine is able to be released from silica-chitosan-sodium alginate microcapsules at pH condition 6,5 better than normal pH condition at 7,4, so the 2% chlorhexidine microencapsulation has the potency to be used as a better root canal drug.

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1. Sodium Alginate as a Controlled Release Microencapsulation 2% Chlorhexidine for Dressing Alternative of Root Canal. Thesis. 2011
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3. Clustering Silica Microcapsule. J Material Kedokteran Gigi.1(2)(2012): 92-9

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